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Environmental DNA metabarcoding: transforming how we survey animal and plant communities

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Abstract

The genomic revolution has fundamentally changed how we survey biodiversity on earth. High-throughput sequencing ('HTS') platforms now enable the rapid sequencing of DNA from diverse kinds of environmental samples (termed 'environmental DNA' or 'eDNA'). Coupling HTS with our ability to associate sequences from eDNA with a taxonomic name is called 'eDNA metabarcoding' and offers a powerful molecular tool capable of non-invasively surveying species richness from many ecosystems. Here, we review the use of eDNA metabarcoding for surveying animal and plant richness, and the challenges in using eDNA approaches to estimate relative abundance. We highlight eDNA applications in freshwater, marine, and terrestrial environments, and in this broad context, we distill what is known about the ability of different eDNA sample types to approximate richness in space and across time. We provide guiding questions for study design and discuss the eDNA metabarcoding workflow with a focus on primers and library preparation methods. We additionally discuss important criteria for consideration of bioinformatic filtering of data sets, with recommendations for increasing transparency. Finally, looking to the future, we discuss emerging applications of eDNA metabarcoding in ecology, conservation, invasion biology, biomonitoring, and how eDNA metabarcoding can empower citizen science and biodiversity education.

Introduction

Anthropogenic influences are causing unprecedented changes to the rate of biodiversity loss and, consequently, ecosystem function (Cardinale *et al.* 2012). Accordingly, we need rapid biodiversity survey tools for measuring fluctuations in species richness to inform conservation and management strategies (Kelly *et al.* 2014). Multi-species detection using DNA derived from environmental samples (termed 'environmental DNA' or 'eDNA') using high-throughput sequencing ('HTS') (Box 1), is a fast and efficient method to survey species richness in natural communities (Creer *et al.* 2016). Bacterial and fungal taxonomic richness (i.e., richness of microorganisms) is routinely surveyed using eDNA metabarcoding and is a powerful complement to conventional culture-based methods (e.g., Caporaso *et al.* 2011; Tedersoo *et al.* 2014). Over the last decade, it has been recognized that animal and plant communities can be surveyed in a similar fashion (Taberlet *et al.* 2012b; Valentini *et al.* 2009).

Many literature reviews summarize how environmental DNA (eDNA) can be used to detect biodiversity, but they focus on single species detections, richness estimates from community DNA (see Box 1 for definition for how this differs and can be confused with eDNA), or general aspects of using eDNA for detection of biodiversity in a specific field of study (Table S1). To compliment these many recent reviews, here we concentrate on four aspects: a summary of eDNA metabarcoding studies on animals and plants to date, knowns and unknowns surrounding the spatial and temporal scale of eDNA information, guidelines and challenges for eDNA study design (with a specific focus on primers and library preparation), and emerging applications of eDNA metabarcoding in the basic and applied sciences.

Surveying species richness and relative abundance with eDNA metabarcoding

Conventional physical, acoustic, and visual-based methods for surveying species richness and relative abundance have been the major ways we observe biodiversity, yet they are not without limitations. For instance, despite highly specialized identification by experts, in some taxonomic groups identification errors are common (Bortolus 2008; Stribling *et al.* 2008). Conventional physical methods can also cause destructive impacts on the environment and to biological communities (Wheeler *et al.* 2004), making them difficult to apply in a conservation context. Furthermore, when a species' behavior or size makes it difficult to survey them (e.g. small bodied or elusive species), conventional methods can require specialized equipment or species-specific observation times, thus making species richness and relative abundance estimates for entire communities intractable (e.g., many amphibians and reptiles, Erb *et al.* 2015; Price *et al.* 2012). These reasons highlight the continued need to develop improved ways to survey global biodiversity, and the unique ways eDNA metabarcoding can complement conventional methods.

Species richness: eDNA metabarcoding compared with conventional methods

Environmental DNA metabarcoding can complement (and overcome the limitations of) conventional methods by targeting different species, sampling greater diversity, and increasing the resolution of taxonomic identifications (Table 1). For example, Valentini *et al.* (2016) demonstrated that, for many different aquatic systems, the number of amphibian species detected using eDNA metabarcoding was equal to or greater than the number detected using conventional methods. When terrestrial hematophagous leeches were used as collectors of eDNA (blood of hosts), endangered and elusive vertebrate species were detected using eDNA metabarcoding and served as a valuable complement to camera trap surveys in a remote geographic region (Schnell *et al.* 2015b). In plants, Kraaijeveld *et al.* (2015) demonstrated that eDNA metabarcoding of

filtered air samples allowed pollen to be identified with greater taxonomic resolution relative to visual methods.

The ways that eDNA can complement and extend conventional surveys are promising, but the spatial and temporal scale of inference is likely to differ between conventional and molecular methods. For example, in a river Deiner *et al.* (2016) showed on a site by site basis that the eDNA metabarcoding method resulted in higher species detection compared to a conventional physical-capture method (i.e., kicknet sampling) (Table 1). However, eDNA in this case may have detected greater species richness at a site not because the species themselves are present, but rather because their DNA has been transported from another location upstream, creating an inference challenge in space and time for eDNA species detections. Therefore, research is needed to understand the complex spatiotemporal dynamics of the various eDNA sample types (Fig 1), which at present we know very little about. In addition, all sampling methods have inherent biases caused by their detection probabilities. Detection probabilities often vary by species, habitat, and detection method (e.g., the mesh size of a net or a primer's match to a target DNA sequence) and use of bias-corrected species richness estimators will be important to account for these biases when conducting statistical comparisons between the outcomes in measured richness (Gotelli & Colwell 2011; Olds *et al.* 2016).

Future methodological comparisons could also benefit from a quantitative ecological approach in the design of sampling by matching sample effort and scope of sampling between eDNA and conventional methods. Multimethod species distribution modeling or site occupancy modeling is one example for how this can be achieved and has been demonstrated in cases comparing qPCR for a single species and conventional methods (Hunter *et al.* 2015; Rees *et al.* 2014a; Schmelzle & Kinziger 2016; Schmidt *et al.* 2013), but rarely for eDNA metabarcoding

(Ficetola *et al.* 2015). Thus, we expect the robustness of eDNA metabarcoding to reveal species richness estimates for animals and plants will be improved by coupling distribution or occupancy modeling with studies to determine the scale of inference in space and time for an eDNA sample (Fig. 1).

Species relative abundance: eDNA metabarcoding compared with conventional methods

Estimating a species' relative abundance using eDNA metabarcoding is an intriguing possibility. Here we focus on the evidence from animals in aquatic systems. Controlled studies based on detection of a single animal species in small ecosystems, such as in aquaria and mesocosms (e.g., Minamoto *et al.* 2012; Moyer *et al.* 2014; Pilliod *et al.* 2013; Thomsen *et al.* 2012a), in natural freshwater systems (e.g., Doi *et al.* 2017; Lacoursière-Roussel *et al.* 2016a) and marine environments (Jo *et al.* 2017; Yamamoto *et al.* 2016) demonstrate that eDNA can be used to measure relative population abundance with a species specific primer set and qPCR. While many more controlled experiments are needed in all ecosystems to determine the relationship of abundance to copy number observed in qPCR, evidence thus far from water samples signifies that eDNA contains information about a species' relative abundance.

Overall, ascertaining abundance information using metabarcoding of eDNA for whole communities still lacks substantial evidence, but some studies in aquatic environments have shown positive relationships with between the relative number of reads and relative or rank abundance estimated with conventional methods. Evans *et al.* (2016) showed in a mesocosm setting that relative abundance of individuals and biomass was correlated with relative read abundance in mesocosms containing fishes and an amphibian. In a natural lake, Hänfling *et al.* (2016) found that the rank abundance derived from long-term monitoring was correlated with read abundance for fish species, and positively correlated with gillnet surveys conducted at the

same time as eDNA sampling. In deep sea habitats, Thomsen *et al.* (2016) found that when reads for fish were pooled to the taxonomic rank of families, there was a correlation with relative abundance of individuals and biomass captured in trawls. While these examples are promising, not all studies support such findings (e.g., Lim *et al.* 2016).

Challenges to accurate abundance estimation through eDNA metabarcoding stem from multiple factors in the field and the lab (Kelly 2016). In the field, the copy number of DNA arising from an individual in an environmental sample is influenced by the characteristics of the 'ecology of eDNA' (e.g., its origin, state, fate, and transport) (Barnes & Turner 2016). Because different animal and plant species are likely to have different rates of eDNA production or 'origin' (Klymus *et al.* 2015), exhibit different 'transport' rates from other locations (Civade *et al.* 2016; Deiner & Altermatt 2014), or stability or 'fate' of eDNA in time (Bista *et al.* 2017; Yoccoz *et al.* 2012), eDNA in an environmental sample could be inconsistent relative to a species' true local and current abundance. Therefore, continued research on how the origin, state, fate, and transport of eDNA influences estimates of relative abundance is needed before we can understand the error this may generate in our ability to estimate abundance.

In the lab, primer bias driven by mismatches with their target have been shown to skew the relative abundance of amplified DNA from mock communities (Elbrecht & Leese 2015; Piñol *et al.* 2015). Similarly, the same mechanism could alter the relative abundance of a species' DNA amplified from eDNA (Fig. 2). Primer bias results in an increased variance in abundance of reads observed relative to their true abundance in an environmental sample (Fig.2). Another source of error is related to library preparation methods. Analysis of mock communities has shown that amount of subsampling during processing steps can drive the loss of rare reads (Leray & Knowlton 2017) and likely occurs for eDNA samples as well (Shelton *et al.* 2016). The

164 combination of primer bias and library preparation procedures alone could cause a large variance
165 in reads observed for any given species and could prevent rare species detection altogether (Fig
166 2). Technical approaches and potential solutions to alleviate primer bias and alternative library
167 preparation methods are discussed in the "Challenges in the field, in the laboratory, and at the
168 keyboard" section. While in the end, it may be that eDNA metabarcoding is not the most
169 accurate method for simultaneously measuring the relative abundance for multiple species from
170 eDNA, researchers should consider whether the eDNA metabarcoding method is accurate
171 enough for application in a particular study or an applied setting. Other methods such as capture
172 enrichment are being examined and are promising because they avoid PCR and hence the bias
173 this may cause, but they do require extensive knowledge of the biodiversity to design targeted
174 gene capture probes and they come with a greater costs for analysis (Dowle *et al.* 2016). Future
175 studies comparing qPCR, eDNA metabarcoding, and capture enrichment will be beneficial to
176 determine which method yields accurate estimates of relative abundance from eDNA.
177 Before ruling out the plausibility entirely, in the short term, simulations could certainly
178 be used to test the effects of technical laboratory issues and account for the ecology of eDNA to
179 decipher under what conditions reliable estimates for abundance can be achieved from eDNA
180 metabarcoding. Promising steps in this direction have been investigated through simulation to
181 learn the nature of how datasets deliberately "noised" conform to neutral theory parameters in
182 estimation of rank abundance curves (Sommeria-Klein *et al.* 2016). Results from simulations
183 studies such as this could then be used to inform mock community experiments and test
184 hypotheses (e.g., type of error distribution expected) under realistic semi-natural environments.
185
186 **Ecosystems, their sample types and known scales of inference in space and time**

187 *Freshwater ecosystems*

188 Environmental DNA metabarcoding of different sample types has been highly successful
189 in obtaining species richness estimates for animals in aquatic systems (Fig.1, Table 1). In one of
190 the first seminal studies, Thomsen and colleagues (2012a) used surface water from lakes, ponds,
191 and streams in Denmark to demonstrate that eDNA contained information about aquatic
192 vertebrate and invertebrate species known from the region. However, there are a notable lack of
193 eDNA metabarcoding studies assessing living aquatic plant communities, and this remains an
194 open area for further research.

195 Mounting evidence suggests that the spatial and temporal scale of inference for eDNA
196 sampled from surface water differs for rivers and lakes (Fig. 1). Specifically, river waters
197 measure species richness present at a larger spatial scale (Deiner *et al.* 2016) compared to eDNA
198 in lake surface waters (Hänfling *et al.* 2016). Differences between lake and river eDNA signals
199 may be due to the transport of eDNA over larger distances in rivers compared to longer retention
200 times of water in lake systems (Turner *et al.* 2015). However, lakes and ponds with river and
201 surface runoff inputs, combined with lake mixing or stratification, may serve as eDNA sources
202 for catchment level terrestrial and aquatic diversity estimates similar to rivers (Deiner *et al.*
203 2016). No studies to date have estimated the sources of eDNA in surface water from a lake's
204 catchment and related it to the diversity locally occurring in the lake. However, ancient DNA
205 from sediment cores in lakes (sedaDNA) has been used to determine historical plant (e.g., Pansu
206 *et al.* 2015b; Parducci *et al.* 2013) and livestock communities (Giguet-Covex *et al.* 2014), thus
207 indicating that lakes do receive DNA from species in their catchments which can be incorporated
208 into their sediments. For a more extensive review of sedaDNA being used to reconstruct past
209 ecosystems see Pederson *et al.* (2015) and Brown and Blois (2016).

Most often, species richness estimates generated from eDNA in surface waters of lakes and rivers reflects recent site biodiversity, while those from eDNA found in surface sediments may reflect a temporally extended accumulation of eDNA. For example, Shaw *et al.* (2016) compared estimates of fish species richness from water and surface sediment samples. Generally they found species were detected in both samples, but estimates of species richness from water samples were in better agreement with the species physically present at the time of sampling. The temporal scale of inference in surface sediments is largely unknown and needs further examination (Fig. 1).

In addition to surface freshwater (~1%), groundwater (~30%) and ice (~69%) comprise much of Earth's freshwater (Gleick 1993). While the other freshwater habitats far surpass the amount of surface water, their extant biodiversity is rather poorly described (Danielopol *et al.* 2000). Groundwater is known to harbor a wide range of specialist taxa which are difficult to assess using conventional survey methods due to the inaccessibility of these habitats (Danielopol *et al.* 2000). Groundwater micro-organism metabarcoding studies have shown high fungal (Sohlberg *et al.* 2015) and bacterial (Kao *et al.* 2016) diversity, and there are examples of species-specific studies on the cave-dwelling amphibian *Proteus anguinus* (e.g., Gorički *et al.* 2017; Vörös *et al.* 2017). However, there is a clear lack of eDNA metabarcoding studies that could shed light on the diversity of a wide range of macro-organisms known to inhabit groundwater; including turbellarians, gastropods, isopods, amphipods, decapods, fishes and salamanders. The spatiotemporal scale of inference of eDNA samples from groundwater is currently unknown. Surveying eDNA in systems with knowledge of the complex hydrology and interactions between surface and ground water will be interesting places to start to reveal the scale of inference for eDNA surveys for these environments.

233 Environmental DNA found in sediment cores and ice core sediments generally reflects a
234 historical biodiversity sample (Fig. 1) and is more commonly used as a source of ancient DNA
235 (Willerslev *et al.* 2007). To date animal and plants surveyed from lake sediment cores suggest
236 that information about terrestrial and aquatic communities can be estimated as far back as 6 to
237 12.6 thousand years before present (Giguet-Covex *et al.* 2014; Pedersen *et al.* 2016), whereas
238 eDNA from sediments in ice cores have successfully been used to reconstruct communities as far
239 back as 2000 years before present (Willerslev *et al.* 1999). The spatial scale of inference for
240 sediment samples types has not been tested, but when samples from multiple locations are
241 combined, large areas can be surveyed for the past presence of species (Anderson-Carpenter *et*
242 *al.* 2011). For modern communities, snow has served as a viable sample type and enabled a local
243 survey of wild canids in France (Valiere & Taberlet 2000). Environmental DNA metabarcoding
244 of water from glacial runoff will also likely be a valuable tool to survey animal and plant
245 richness living in glacial and subglacial habitats, which are undergoing dramatic change because
246 of climate warming (Giersch *et al.* 2017).

247 *Marine ecosystems*

248 The use of eDNA metabarcoding is often described as challenging in marine ecosystems,
249 due to the potential dilution of eDNA in large volumes of water and additional abiotic factors
250 (salinity, tides, currents) that likely impact eDNA transport and degradation (Foote *et al.* 2012;
251 Port *et al.* 2016; Thomsen *et al.* 2012b), not to mention the logistics involved in undertaking
252 such surveys. Nonetheless, eDNA metabarcoding surveys of marine fish from coastal water
253 samples have demonstrated that eDNA can detect a greater taxonomic diversity compared to
254 conventional survey techniques (Table 1), while simultaneously improving detection of rare and
255 vagrant fish species, and revealing cryptic species otherwise overlooked by visual assessments

(O'Donnell *et al.* 2017; Port *et al.* 2016; Thomsen *et al.* 2012b; Thomsen *et al.* 2016). Marine mammals have been surveyed with acoustic surveys and eDNA metabarcoding, and here the conventional acoustic methods detected a greater species richness (Foote *et al.* 2012). Nevertheless, this study used low sample volumes compared to other marine studies (15 – 45 mL vs. 1.5 – 3.0 L) and the authors concluded that larger sample volumes would likely lead to greater similarity between eDNA and conventional methods. In Monterey Bay, California, water sampled from depths less than 200 m or greater 200 m were used to detect marine mammals such as seals, dolphins, and whales in addition to many fishes and sharks (Andruszkiewicz *et al.* 2017). The taxonomic groups detected were spatially explicit and were found more or less in water associated with their expected habitat.

Longitudinal transport of animal and plant eDNA in marine environments is not well studied. But, similar to freshwater sediment cores from lakes, vertical transport into marine sediments is likely to preserve a large proportion of eDNA from particulate organic matter or eDNA that has become directly adsorbed onto sediment particles. This absorption shields nucleotides from degradation (particularly oxidation and hydrolysis) and facilitates long-term preservation of genetic signals over potentially large spatiotemporal scales (Fig. 1). Marine sediment eDNA concentrations have been shown to be three orders of magnitude higher than in seawater eDNA (Torti *et al.* 2015) and eDNA from both ancient and extant communities is typically recovered (Lejzerowicz *et al.* 2013). Similar to lake sediments, marine sediments can accumulate genetic information from both terrestrial and pelagic sources (Torti *et al.* 2015).

Marine sediments are difficult to sample because of the logistical effort involved in obtaining samples, which often requires ship time and specialized coring equipment. Even though much work remains to be done to understand the spatiotemporal scale of inference for

279 marine sediment cores, comparisons between eDNA and environmental RNA (eRNA)
280 metabarcoding are hypothesized to allow inference between present and past diversity.
281 Environmental RNA is thought to be only available from live organisms in the community, thus
282 the comparison between eDNA and eRNA has been investigated. In applied settings, eDNA
283 metabarcoding of surface sediments has revealed benthic impacts of aquaculture for Atlantic
284 salmon farming on short spatial scales using both eDNA and eRNA (Pawlowski *et al.* 2014).
285 Guardiola *et al.* (2016) showed through a comparison of eDNA and eRNA that spatial trends in
286 species richness from these two sources were similar, but that eDNA detected higher diversity.
287 Overall, the fate, transport, and decomposition of animal and plant eDNA in marine
288 environments is poorly known compared to other environments, and there is pressing need for
289 further studies.

290 *Terrestrial and aerial ecosystems*

291 Environmental DNA from terrestrial sediment cores is a valuable tool for investigating
292 past environments and reconstructing animal and plant communities (Fig. 1, Haouchar *et al.*
293 2014; Jørgensen *et al.* 2012; Willerslev *et al.* 2003). Animal remains also provide opportunities
294 to reconstruct past trophic relationships. For example, eDNA metabarcoding of pellets in
295 herbivore middens have been used to identify species in ancient animal and plant communities
296 (Fig. 2, Murray *et al.* 2012) and DNA traces from microplant fossils within coprolites were used
297 to reconstruct former feeding relationships in rare and extinct birds (Wood *et al.* 2012). Again
298 here, the recent reviews of Brown & Blois (2016) and Pedersen *et al.* (Pedersen *et al.* 2015)
299 provide a more extensive overview for how ancient DNA is used to uncover past animal and
300 plant communities.

In modern environments, eDNA isolated from top soils has been used to characterize biodiversity in earthworms (Bienert *et al.* 2012; Pansu *et al.* 2015a), invertebrates (McGee & Eaton 2015), plants (Taberlet *et al.* 2012c; Yoccoz *et al.* 2012) and vertebrate species (Andersen *et al.* 2012). In what is perhaps the most comprehensive analysis using eDNA metabarcoding for any environment, Drummond *et al.* (2015) simultaneously surveyed all three domains of life in top soil using PCR primers that amplified five different metabarcoding regions, thus demonstrating the power of this method for assessing total richness for an area. However, the spatial scale of inference for many terrestrial eDNA samples is an open question (Fig. 1). Research on the time scale of inference for DNA in top soil suggests that long fragments of DNA break down quickly, but short fragments remain detectable for days to years after the presence of the species (Taberlet *et al.* 2012c; Yoccoz *et al.* 2012). Thus, the fragment length amplified can change the temporal resolution of a soil sample.

There are many additional sources for eDNA sampling besides soil in terrestrial ecosystems. For animals, blood meals from leeches (Schnell *et al.* 2012) and carrion flies (Calvignac-Spencer *et al.* 2013) have been used to survey mammal diversity. Saliva on browsed twigs was tested as a source of eDNA to survey ungulates (Nichols *et al.* 2012) and on predated eggs and carcasses of ground-nesting birds to discover predators or scavengers (Hopken *et al.* 2016). DNA extracted from spider webs has also been used to detect spiders and their prey (Xu *et al.* 2015). For plants, pollen within honey has revealed honey bee foraging preferences (De Vere *et al.* 2017; Hawkins *et al.* 2015). Craine *et al.* (2017) surveyed dust from indoor and outdoor environments throughout the United States and found that plant DNA from known allergens was almost twice as high outdoor compared with indoor environments. In addition to allergen detection from pollen, there remain many potential applications of dust eDNA to assess

animal species richness. Fecal DNA has also been used as a source of eDNA to assess diet composition, but most studies utilizing this source of eDNA are focused on single species detections and population genetic inferences (see review from Rodgers & Janečka 2013) and are not necessarily using eDNA sources from fecal DNA to estimate species richness of terrestrial communities. Boyer *et al.* (2015) proposed that surveys of feces from generalist predators can act as 'biodiversity capsules' and analysis of this eDNA source should give rise to biodiversity surveys for prey communities in landscapes. While all of these sources are available, most of these sample types (e.g., leaves from a tree, fecal pellets, spider webs, and dust) do not have a known scale of inference in space and time. A single sample of eDNA from these sources is not likely to confirm species richness for more than a local scale, but combination of multiple sample sources (e.g., leaves, fecal pellets, and spider webs throughout a park) and sampled over time may allow for spatial and temporal estimates of terrestrial species richness.

Surveys of airborne eDNA have placed greater emphasis on the detection of bioaerosols that cause infection or allergic responses in animals and plants (West *et al.* 2008). For example, Kraaijeveld (2015) investigated airborne pollen that can cause hay fever and asthma in humans and showed that the source of allergenic plant pollen could be identified more accurately using eDNA from plant pollen filtered from the air compared to microscopic identification. A particularly interesting area for further research is to gain an understanding of the scale of inference for air samples in space and time (Fig. 1). While plant eDNA can be ascertained, surveying other species such as birds and insects from aerial eDNA sources has not been tested to our knowledge.

Challenges in the field, in the laboratory, and at the keyboard

Despite the obvious power of the approach, eDNA metabarcoding is affected by a host of precision and accuracy challenges distributed throughout the workflow in the field, in the laboratory, and at the keyboard (Thomsen & Willerslev 2015). Following study design (e.g., hypothesis/question, targeted taxonomic group, *etc.* Fig. 3), the current eDNA workflow consists of three components: field, laboratory, and bioinformatics. The field component consists of sample collection (e.g., water, sediment, air) that is preserved or frozen prior to DNA extraction. The laboratory component has four basic steps: 1) DNA is concentrated (if not done in the field) and purified, 2) PCR is used to amplify a target gene or region, 3) unique nucleotide sequences called 'indexes' (also referred to as 'barcodes') are incorporated using PCR or are ligated onto different PCR products, creating a 'library' whereby multiple samples can be pooled together, 4) pooled libraries are then sequenced on a high-throughput machine (most often the Illumina HiSeq or MiSeq platform). The final step after laboratory processing of samples is to computationally process the output files from the sequencer using a robust bioinformatics pipeline (Fig. 3, Box 2). Below we emphasize the important and rapidly evolving aspects of the eDNA metabarcoding workflow and give recommendations for ways to reduce error.

In the field

As for any field study, the study design is of paramount importance (Fig. 3, Box 2), since it will impact the downstream statistical power and analytical interpretation of any eDNA metabarcoding dataset. For example, sampling effort and replication (especially biological), are positively correlated with the probability of detecting the target taxa (Furlan *et al.* 2016; Willoughby *et al.* 2016). Despite the extensive evidence of the occurrence of macro-organism DNA in the environment, our fundamental understanding of what 'eDNA' is from any environmental sample is still lacking. For an illustration of this challenge, we summarize what is

known about eDNA in freshwater environments. The current state-of-the-art relies on the fact that we can access eDNA by precipitating DNA from small volumes of water samples (e.g., 15 mL, Ficetola *et al.* 2008), or filter eDNA from the water column using a variety of filter sizes (0.22 µm and upwards) (Rees *et al.* 2014b). Filtration protocols lead to a working hypothesis that aqueous eDNA is either derived from cellular or organellar sources (e.g., mitochondria, Lacoursière-Roussel *et al.* 2016b; Turner *et al.* 2014; Wilcox *et al.* 2015), and precipitation protocols suggest extracellular sources (Torti *et al.* 2015). It is clear that at least some freshwater eDNA comes from intact cellular or organellar sources because it has recently been demonstrated to be available in the genomic state (Deiner *et al.* 2017b). Thus, eDNA in water exists in both un-degraded and degraded forms (Deiner *et al.* 2017b). However, continued research on the origin, state, and fate of eDNA will greatly inform numerous strategies regarding its acquisition (filtering, replication, sample volumes and spatial sampling strategies) (Barnes & Turner 2016). Many methods for solving current challenges of false negatives (e.g., use of biological replicate sampling, improved laboratory methods) and false positives (e.g., use of negative controls) in the field are explored in a recent review (Goldberg *et al.* 2016), we therefore refer readers to this review rather than treat those topics in-depth here.

In the laboratory

There are a number of recent studies that focus on the capture, preservation, and extraction of eDNA and the literature reviewed therein summarizes the important considerations and trade-offs that should be tested before a large scale study is conducted (e.g., Deiner *et al.* 2015; Renshaw *et al.* 2015; Spens *et al.* 2017). Rather than reiterate those aspects here, we focus on primer choice and library preparation. For animal and plant studies, PCR primers most often

target mitochondrial or plastid loci or nuclear ribosomal RNA genes (Table S2). The standard barcoding markers defined by the Consortium for the Barcode of Life (CBOL) are Cytochrome c oxidase subunit I (COI or cox1), for taxonomical identification of animals (Hebert *et al.* 2003), and a 2-loci combination of *rbcL* and *matK* as the plant barcode (Hollingsworth *et al.* 2009) with ITS2 also suggested as valid plant barcode marker (Chen *et al.* 2010). However, there are limitations for using the standard barcoding markers in macro-organism eDNA metabarcoding. Specific to COI, other DNA regions are commonly used because not all taxonomic groups can be differentiated to species equally well (Deagle et al. 2014) and because it is challenging to design primers in this gene for a length that is suitable for short amplicon analysis, but some regions have been identified (Leray *et al.* 2013). The most common alternative markers used are mitochondrial ribosomal genes such as 12S and 16S or protein coding genes such as Cytochrome B (Table S2). Specific to the plant barcoding loci, the 2-loci primarily used for barcoding plants can be independently generated, but is not always possible to recover which fragment from each gene is associated with each other in an eDNA sample; rendering species identification using the standard plant barcode challenging. Bioinformatic methods can help resolve these situations to some extent, and may work when diversity is low in a sample (Bell *et al.* 2016). Therefore, often one or different markers are used (e.g., P6 loop of the trnL intron (Sønstebø *et al.* 2010; Taberlet *et al.* 2007)) (Table S2).

Additionally, some highly-evolving non-coding loci, such as ITS rRNA, are used (Table S2), but these markers do not always allow for the construction of alignments to determine MOTUs during data analysis because they have intragenomic variation that complicates their use in biodiversity studies (plant ITS rRNA may be an exception (Bell *et al.* 2016)). For these loci, an unknown environmental sequence is often discarded unless it has an exact database match

reducing a dataset to only known and sequenced biodiversity. Due to these factors, other metabarcoding loci such as 18S rRNA genes may be more appropriate (e.g., in studies of marine invertebrates, Bik *et al.* 2012), especially if phylogenetic analysis is needed to narrow down taxonomic assignments and circumvent database limitations (Box 3).

Once the locus or loci are chosen, primers are then designed based on the taxonomic group(s) of interest within a study, and the need for broad (multiple phyla) vs. narrow (single order) coverage to test study-specific hypotheses (Fig. 3). When choosing previously designed primers (Table S2) or when designing new primers it is important to perform rigorous testing, *in silico*, *in vitro* and *in situ* to infer their utility for metabarcoding eDNA in a new study system (Elbrecht & Leese 2017; Freeland 2016; Goldberg *et al.* 2016). Amplicon size is also an important consideration because there may be a trade-off in detection with amplicon length (e.g., short fragments are more likely to amplify). However, short fragments may persist longer in the environment and increase the inference in space or time that can be made from an environmental sample (Bista *et al.* 2017; Deagle *et al.* 2006; Jo *et al.* 2017; Yoccoz *et al.* 2012). Additionally, use of more than one locus for a target group can allow for tests of consistency between loci and increase stringency of detection for any species (Evans *et al.* 2017).

Once primers are designed and PCR products are amplified, eDNA metabarcoding relies on multiplexing large numbers of samples on HTS platforms in order to make the tool cost effective. Illumina (MiSeq and HiSeq) sequencing platforms at the moment outperform other models for accuracy (Loman *et al.* 2012) and multiplexing samples is usually achieved by the incorporation of sample-specific nucleotide indices and sequencing adapters during PCR amplification. However, multiplexing creates opportunities for errors and biases. In this facet of the workflow it is important to avoid methods that induce sample specific biases in amplification

(O'Donnell *et al.* 2016) and to reduce the potential for index crossover, or "tag jumping" (see Box 2) (Schnell *et al.* 2015a). To address these issues, Illumina has developed a two-step PCR protocol using uniformly tailed primers across samples for the first step and sample specific indexes for the second PCR, which could reduce bias related to index sequence variations (Berry *et al.* 2011; Miya *et al.* 2015; O'Donnell *et al.* 2016). Regardless of the strategy employed extreme care is needed to ensure primer quality control (e.g., both use of small aliquots from stocks as well as proper cleaning of PCR amplified products to remove indexing primers after amplification (Schnell *et al.* 2015a). When a species detection is suspected as highly unlikely in a sample, single-species quantitative PCR (qPCR) can be used to verify its presence from the same eDNA sample because qPCR does not suffer from the same technical sources of error. Additional suggestions for dealing with multiplexing artifacts are suggested in Box 2 under "abundance filtering".

In addition, both positive and negative controls must be used in the lab to ensure sample integrity (Fig. 3). Use of positive control samples (either from pooled DNA extracts derived from tissue at the PCR stage, or used at the extraction stage alongside that of eDNA samples) can help evaluate sequencing efficiency and multiplexing errors in the eDNA metabarcoding workflow (Hänfling *et al.* 2016; Olds *et al.* 2016; Port *et al.* 2016). Careful thought in the construction of the mock community is needed. Typically, species not expected in the study area are used (Olds *et al.* 2016; Thomsen *et al.* 2016) such that if there is contamination during the workflow their reads can be identified, removed and serve as a control for detecting contamination when it occurs.

Negative controls should be introduced at each stage of lab work (i.e., filtration - if done in the lab, extraction, PCR, and indexing). We recommend that an equivalent amount of

technical replication should be used on negative and positive controls as that carried out on actual samples (Ficetola *et al.* 2015). Furthermore, it is becoming important that negative controls are sequenced regardless of having detectable amounts of DNA because contamination can be below detection limits of quantification and sequences found in these controls can be used to detect de-multiplexing errors or used in statistical modeling to rule out false positive detections (Olds *et al.* 2016).

Finally, an important but often neglected consideration for the eDNA metabarcoding workflow, is the identification of technical artifacts that arise independently of true biological variation. For example, recently in a study focused on bacterial biodiversity using the 16S locus it was shown that a run effect can be confounded with a sample effect if it is not accounted for (e.g., by splitting sample groups across multiple Illumina runs, Chase *et al.* 2016); however, it remains to be seen whether such technical artifacts are also prevalent for loci used for metabarcoding plant and animals from eDNA (COI, 18S, ITS, etc.) and more research is needed. Until then, careful thought into how samples are pooled and run on a sequencer seems warranted in order to not confound the hypotheses being tested.

At the keyboard

Bioinformatic processing of high throughput sequence datasets requires the use of UNIX pipelines (or graphical wrappers of such tools, Bik *et al.* 2012). Metabarcoding of animal and plant community DNA is comprehensively outlined in Coissac *et al.* (2012). Below and in Box 3 we highlight the common practices to community DNA metabarcoding and deviations for studies focusing on macro-organism eDNA metabarcoding.

Bioinformatic pipelines and parameters must be carefully considered (Box 2) and it is important to work with a knowledgeable computational researcher to understand how processing

485 can impact the biological results and conclusions. Before computationally processing an eDNA
486 metabarcoding dataset, perhaps the strongest message from Coissac *et al.* (2012) is to identify
487 the differences between the analysis of data derived from microbial and macro-organismal
488 groups. Since microbial ecologists have been inspired to use sequence-based identification of
489 taxa over the past 40 years (Creer *et al.* 2016), the range of software solutions to analyze
490 microbial metabarcoding datasets is unsurprisingly extensive (Bik *et al.* 2012). Perhaps more
491 importantly, a number of established and maintained databases exist featuring many of the
492 commonly used microbial taxonomic markers for prokaryotes (Cole *et al.* 2009), microbial
493 eukaryotes (Guillou *et al.* 2013; Pruesse *et al.* 2007; Quast *et al.* 2012) and fungi (Abarenkov *et*
494 *al.* 2010), meaning that microbial datasets can be analyzed and taxonomic affiliations established
495 are established in a straight forward way.
496 For macro-organism communities, pre-processing and initial quality control of eDNA
497 metabarcoded data sets is not different from that of microbial datasets and can be acquired using
498 packages developed either for microbial (Caporaso *et al.* 2010), or macro-organism data (Boyer
499 *et al.* 2016), but taxonomic assignment will require a robust dataset of locus-specific reference
500 sequences and the associated taxonomic data from a reference database (Coissac *et al.* 2012)
501 (Box 3). Currently the two most common reference sources for macro-organisms are NCBI's
502 nucleotide database (Benson *et al.* 2013) and the Barcode of Life Database (Ratnasingham &
503 Hebert 2007). The utility and taxonomic breadth of these databases can be enhanced by the
504 creation of custom-made or hybrid databases, with the obvious additional workload and cost
505 depending on the number of focal taxa missing from current data sources. Recently, Machida *et*
506 *al.* (2017) have assembled and proposed metazoan mitochondrial gene sequence datasets that can
507 be used for taxonomic assignment for environmental samples. While these datasets do not

account for future growth, their methods could be repeated at the time of any new study to generate a custom reference dataset for taxonomic assignment.

Macro-organism eDNA metabarcoding datasets are associated with advantages compared to microbial datasets because the number of taxa in any survey will be comparatively low, reducing the computational time needed for taxonomic annotation. Moreover, the species delimitation concepts and taxonomic markers associated with macro-organisms are well-developed (de Queiroz 2005) and can even be used to analyze population genetic structure (Sigsgaard *et al.* 2016; Thomsen & Willerslev 2015), or delimit species boundaries (Coissac *et al.* 2012; Hebert *et al.* 2003; Tang *et al.* 2014). Reliance on the vast knowledge we have for animal and plant taxonomy and biogeography is a distinct advantage for eDNA metabarcoding because of the independent test that it provides to calibrate and test the tool for its precision and accuracy (Deiner *et al.* 2016).

Data archiving for transparency

As eDNA applications continue to develop, all procedures used in the field, lab, and during bioinformatic data processing require a strong commitment to transparency on the part of researchers (Nekrutenko & Taylor 2012). Here, we outline best practices for eDNA metabarcoding studies of macro-organisms, following on from well-established standards in the fields of microbiology and genomics (Yilmaz *et al.* 2011). First, raw FASTQ files from any HTS run need to be submitted to the Sequence Read Archive (SRA) of NCBI or the European Nucleotide Archive (ENA) and other such public national data bases before publication. Archiving raw data in publicly available databases is common practice in virtually all genomics and transcriptomic studies because it allows studies to be re-analyzed with new computational tools and standards. In fact, archiving raw data is becoming increasingly mandatory at many

evolutionary and ecology biology journals, inclusive of Molecular Ecology. Second, researchers should adhere to minimum reporting standards defined by the broader genomics community, such as the MIMARKS (Minimum information about a marker gene sequence) and MIxS (minimum information about any "x" sequence) specifications (Yilmaz *et al.* 2011). Goldberg *et al.* (2016) have made specific recommendations for upholding these reporting standards specific to eDNA studies (see Table 1 in Goldberg *et al.* 2016).

Third, computational processing of data needs to be reproducible (Sandve *et al.* 2013). For eDNA metabarcoding studies, it is increasingly common to deposit a comprehensive sample mapping file (e.g., formatted in the QIIME tab-delimited style, containing the indexes used for creating libraries so that raw data can be de-multiplexed and properly trimmed) along with MOTU clustering or taxonomic binning of results, and documentation of all bioinformatics commands, in a complementary repository such as Dryad (http://datadryad.org/), GitHub (https://github.com/github), or FigShare (http://figshare.com). Sandve *et al.* (2013) provide 10 rules that can be followed to ensure such reproducibility, and we strongly encourage researchers using eDNA metabarcoding methods to uphold these practices and take advantage of archiving intermediate steps (Box 2) of their analysis for full transparency.

Emerging applications for eDNA metabarcoding

Applications in ecology

Quantifying the richness and abundance of species in natural communities is and will continue to be a goal in many ecological studies. Information about species richness garnered from eDNA is not necessarily different from conventional approaches (Table 1), but the scale, speed, and comprehensiveness of that information is (Fig. 4). For example, Drummond *et al.*

554 (2015) demonstrated the near-complete analysis of biodiversity (e.g., from bacteria to animals
555 and plants) from top soil is possible. Collection of data on this taxonomic scale opens up new
556 opportunities with respect to measuring community composition and turnover across space and
557 time. In addition to estimating species richness, a major area of research in ecology is
558 determining whether observed community changes surpasses acceptable thresholds for certain
559 desired ecosystem functions (Jackson *et al.* 2016). Biodiversity and ecosystem functioning
560 research requires tracking species in multiple taxonomic groups and trophic levels, along with
561 changes in ecosystem function. Environmental DNA metabarcoding has the potential to facilitate
562 biodiversity and ecosystem function research by improving our knowledge of predator/prey
563 relationships, mutualisms such as plant-pollinator interactions, and food webs in highly diverse
564 systems composed of small cryptic species (e.g., De Vere *et al.* 2017; Hawkins *et al.* 2015; Xu *et*
565 *al.* 2015). Knowledge of species co-occurrences and interactions in these instances will
566 additionally foster the study of meta ecosystems and provide data to guide management
567 decisions at the ecosystem scale (Bohan *et al.* 2017). What will remain challenging is moving
568 beyond richness estimates to also obtaining species abundance data (Fig. 2 & 4).
569
570 *Applications in conservation biology*
571 Given the rapid rate at which biodiversity is declining worldwide (Butchart *et al.* 2010), it
572 is critical that we improve the effectiveness of strategies to halt or reverse this loss (Thomsen &
573 Willerslev 2015; Valentini *et al.* 2016). Accordingly, developing tools that enable rapid, cost-
574 effective and non-invasive biodiversity assessment such as eDNA metabarcoding, especially for
575 rare and cryptic species, is paramount (Fig. 4). Improved estimates of the distribution of
576 vulnerable species, and done so non-invasively, would facilitate policy development and allow

577 for efficient targeting of management efforts across habitats (Kelly *et al.* 2014; Thomsen &
578 Willerslev 2015). For example, documenting the presence of threatened species in a habitat can
579 trigger a suite of actions under laws pertaining to biodiversity conservation (e.g., US Endangered
580 Species Act). Frequently, data relevant to policy are derived from monitoring efforts mandated
581 by environmental laws imparting a significant consequence to the data collected (Kelly *et al.*
582 2014).

583 Environmental DNA-based monitoring is likely to be a tremendous boon to often
584 underfunded public agencies charged with compliance to data-demanding laws. Specifically,
585 eDNA metabarcoding will be useful for monitoring communities when many species are of
586 conservation concern. Vernal pools throughout California are a prime example because they
587 contain 20 US federally listed endangered or threatened species of plants and animals.
588 Monitoring species richness with soil and water samples from a habitat such as this would
589 provide a comprehensive sampling method to ascertain needed community data for their
590 conservation and management (Deiner *et al.* 2017a). However, while eDNA metabarcoding may
591 be important for non-invasively gaining access to the distribution of vulnerable species, it cannot
592 be used to differentiate between alive and dead organisms or estimate many demographic
593 parameters important of population viability analysis (Beissinger & McCullough 2002).

594 Quantifying baselines of animal and plant species richness and departures from those
595 baselines, is central to the assessment of environmental impact and conservation (Taylor &
596 Gemmell 2016). The application of eDNA metabarcoding methods to different samples types,
597 which taken together allow inference across time (e.g., surface water and sediment layers from a
598 core in a lake, Fig. 1) provides a unique tool to document local extinctions and long-term
599 changes in ecosystems. Extinction models often rely on and understanding extinction timelines

600 (reviewed in Thomsen & Willerslev 2015). The efficiency of eDNA metabarcoding to track the
601 timing of extinctions associated with previous glacial events has been demonstrated in mammals
602 (Haile *et al.* 2009) and plants (Willerslev *et al.* 2014). Thus, environmental DNA metabarcoding
603 of different sample types from the same site offers an excellent opportunity to better understand
604 the extinction consequences of perturbations and could inform scenario modeling under climate
605 change.
606
607 *Applications in invasion biology*
608 Because one of the first applications of eDNA to macro-organisms was the detection of
609 North American bullfrogs in French ponds (Ficetola *et al.* 2008), the method immediately came
610 to the attention of researchers interested in invasion biology (e.g., Egan *et al.* 2013; Goldberg *et*
611 *al.* 2013; Jerde *et al.* 2011; Takahara *et al.* 2013; Tréguier *et al.* 2014). These initial studies, as
612 well as much ongoing research, continue to be based on species-specific primers, where positive
613 amplification provides occurrence evidence for a particular invasive species. In invasion biology
614 with eDNA, such a targeted approach is referred to as "active" surveillance (Simmons *et al.*
615 2015).
616 On the contrary, eDNA metabarcoding makes it possible to detect the presence of many
617 species simultaneously, including species not previously suspected of being present. This
618 broader untargeted approach is called "passive" surveillance in management applications (Fig. 4)
619 (Simmons *et al.* 2015). On the down side, due to a trade-off in primer specificity, we expect that
620 eDNA metabarcoding may be less sensitive in detecting some species or that the detection rate of
621 a species can change depending on species richness. Adopting a dual approach of passive and
622 active surveillance could be considered in cases where the risk of a new invasion is high, and

where cost effective eradication plans for undesirable species are likely to be successful (Lodge *et al.* 2016).

Avoiding future introductions and reducing the spread of exotic species is paramount in natural resource policy (Lodge *et al.* 2016). Environmental DNA metabarcoding relevant to management includes early detection of incipient invasive populations in the environment, surveillance of invasion pathways, e.g., ballast water of ships (Egan *et al.* 2015; Zaiko *et al.* 2015), and the live bait trade (Mahon *et al.* 2014). While eDNA metabarcoding is not yet routinely used for biosecurity regulation of invasive species or enforcement in many settings, it has the potential to become valuable monitoring tool for biological invasions. An important challenge for the use of eDNA metabarcoding in invasive species detections are false positives and false negatives since both outcomes can trigger action or inaction when not required, causing a potentially large burden on entities responsible for invasive species mitigation and control (Fig. 4). Therefore, continued research to reduce or understand the nature of false positives and false negatives will reduce uncertainty in the tool and facilitate greater adoption.

Applications in biomonitoring

Pollution of air, water, and land resources generated from processes such as urbanization, food production, and mining is one of the many emerging global challenges we are facing in the 21st century (Vörösmarty *et al.* 2010). Determine the origin, transport, and effects of most pollution is challenging because it accumulates through both point sources (e.g., wastewater effluent) and diffused sources related to land-use types (e.g., agriculture or urbanization). In this context, the presence of tolerant or absence of sensitive organisms has been used to determine the consequences of pollution on ecosystem health throughout the world and is termed biological

monitoring or ‘biomonitoring’ (Bonada *et al.* 2006). The extent to which animals and plants have been used in biomonitoring depends on the unique characteristics of the taxonomic group monitored and their relationship to the pollution of interest (Bonada *et al.* 2006; Stankovic *et al.* 2014). Most biomonitoring programs take community composition and often abundance of taxa into account and calculate what is known as a biotic index (Friberg *et al.* 2011). Biotic indices take many forms and are typically surrogates for the impacts of pollution (e.g., SPEAR index for toxicant exposure in water, Liess *et al.* 2008).

Applying eDNA metabarcoding in the context of biomonitoring is a major avenue of research. Metabarcoding of community DNA samples has shown greater sensitivity for detecting cryptic taxa or life stages and can alleviate the problem of identifying damaged specimens of which render morphological tools ineffective (Gibson *et al.* 2014; Hajibabaei *et al.* 2011). These two issues alone are known to create large variances in biotic index estimation (Pfrender *et al.* 2010). Application of eDNA metabarcoding to animals and plants used in biomonitoring requires in-depth testing of conventional survey methods and eDNA-based approaches (Fig. 4), to understand whether species richness estimates derived from the two methods result in a similar measure for the biotic index of interest or whether new biotic indices need to be development that can simultaneously consider both forms of information. Promising steps forward are being made through the DNA AquaNet COST Action (http://dnaqua.net/) which is a consortium of over 26 European union countries and four international partners working together to develop genetic tools for bioassessment of aquatic ecosystems in Europe (Leese *et al.* 2016).

Applications in citizen science and biodiversity education

668 The simplicity of the protocol used to collect environmental samples has created an
669 avenue for citizen scientist programs to be built around surveying for biodiversity using eDNA
670 (Biggs *et al.* 2015). With the development of sample kits from commercial companies
671 specifically used for eDNA analysis (e.g., GENIDAQS, ID-GENE, Jonah Ventures,
672 NatureMetrics, Spygen) there now exists a novel opportunity to engage the public in biodiversity
673 science, which could accompany already established biodiversity events, such as BioBlitz
674 (National Geographic Society). Use of eDNA metabarcoding in this context will likely provide
675 an unprecedented tool for education and outreach about biodiversity, and increase awareness
676 about its decline. Challenges that hinder integration of eDNA metabarcoding in citizen science
677 projects and educational opportunities are the time and costs needed to process samples and user
678 friendly data visualization tools to allow exploration of the data once provided. Thus, finding
679 ways to cut costs and speed up data generation (a goal common for any application of the tool),
680 as well as creation of applications for exploration of data on smart phones and desktops alike is
681 needed to propel the use of eDNA applications in citizen science and education.
682
683 **Conclusions**
684 As the tool of eDNA metabarcoding continues to develop, our understanding regarding
685 the analysis of eDNA from macro-organismal communities, including optimal field, laboratory,
686 and bioinformatics workflows will continue to improve in the foreseeable future. Concurrently,
687 we need to gain a better understanding of the spatial and temporal relationship between eDNA
688 and living communities to improve precision, accuracy, and to enhance the ecological and policy
689 relevance of eDNA (Barnes & Turner 2016; Kelly *et al.* 2014). Ultimately, the errors and
690 uncertainties associated with eDNA metabarcoding studies can often be mitigated by thoughtful

691 study design, appropriate primer choice, and robust sampling and replication: as Murray et al.
692 (2015) emphasize, “no amount of high-end bioinformatics can compensate for poorly prepared
693 samples, artefacts or contamination.”

694 Over time, a loop in which improved eDNA metabarcoding methods reduce uncertainty
695 about the meaning of both positive and negative eDNA detections for a species will in turn
696 generate the motivation for continued improvements and use of eDNA metabarcoding methods.
697 Thus, resulting in the adoption of eDNA metabarcoding as a comparable method for estimating
698 species richness. We predict that over the next decade eDNA metabarcoding of animals and
699 plants will become a standard surveying tool that will complement conventional methods and
700 accelerate our understanding of biodiversity across the planet.

Box 1: Community DNA versus environmental DNA metabarcoding of plants and animals

Terms:

Environmental DNA (eDNA). DNA captured from an environmental sample without first isolating any target organisms (Taberlet *et al.* 2012a). Traces of DNA can be from feces, mucus, skin cells, organelles, gametes or even extracellular DNA. Environmental DNA can be sampled from modern environments (e.g., seawater, freshwater, soil or air) or ancient environments (e.g., cores from sediment, ice or permafrost (e.g., cores from sediment, ice or permafrost, see Thomsen & Willerslev 2015).

Community DNA. DNA is isolated from bulk-extracted mixtures of organisms separated from the environmental sample (e.g., soil or water).

Macro-organism environmental DNA. Environmental DNA originating from animals and higher plants.

Barcoding. First defined by Hebert *et al.* (2003), the term refers to taxonomic identification of species based on single specimen sequencing of diagnostic barcoding markers (e.g., COI, *rbcL*).

Metabarcoding. Taxonomic identification of multiple species extracted from a mixed sample (community DNA or eDNA) which have been PCR amplified and sequenced on a high throughput platform (e.g., Illumina, Ion Torrent).

High Throughput Sequencing (HTS). Sequencing techniques which allow for simultaneous analysis of millions of sequences compared to the Sanger sequence method of processing one sequence at a time.

Community DNA metabarcoding: HTS of DNA extracted from specimens or whole organisms collected together, but first separated from the environmental sample (e.g., water or soil).

Molecular Operational Taxonomic Unit (MOTU): Group identified through use of cluster algorithms and a predefined percent sequence similarity (e.g., 97%) (Blaxter *et al.* 2005).

Since the inception of High Throughput Sequencing (HTS, Margulies *et al.* 2005), the use of metabarcoding as a biodiversity detection tool has drawn immense interest (e.g., Creer *et al.* 2016; Hajibabaei *et al.* 2011). However, there has yet to be clarity regarding what source material is used to conduct metabarcoding analyses (e.g., environmental DNA versus community DNA). Without clarity between these two source materials, differences in sampling, as well as differences in lab procedures, can impact subsequent bioinformatics pipelines used for data processing, and complicate the interpretation of spatial and temporal biodiversity patterns. Here we seek to clearly differentiate among the prevailing source materials used and their effect on downstream analysis and interpretation for environmental DNA metabarcoding of animals and plants compared to that of community DNA metabarcoding.

With community DNA metabarcoding of animals and plants, the targeted groups are most often collected in bulk (e.g., soil, malaise trap, or net), individuals are removed from other sample debris and pooled together prior to bulk DNA extraction (Creer *et al.* 2016). In contrast, macro-organism eDNA is isolated directly from an environmental material (e.g., soil or water) without prior segregation of individual organisms or plant material from the sample and implicitly assumes that the whole organism is not present in the sample. Of course, community DNA samples may contain DNA from parts of tissues, cells, and organelles of other organisms (e.g., gut contents, cutaneous intracellular or extracellular DNA, *etc.*). Likewise, macro-organism

746 eDNA samples may inadvertently capture whole microscopic non-target organisms (e.g.,
747 protists, bacteria, *etc.*). Thus, the distinction can at least partly breaks down in practice.
748
749 Another important distinction between community DNA and macro-organism eDNA is
750 that sequences generated from community DNA metabarcoding can be taxonomically verified
751 when the specimens are not destroyed in the extraction process. Here sequences can then be
752 generated from voucher specimens using Sanger sequencing. Since the samples for eDNA
753 metabarcoding lack whole organisms, no such *in situ* comparisons can be made. Taxonomic
754 affinities can therefore only be established by directly comparing obtained sequences (or through
755 bioinformatically generated operational taxonomic units (MOTUs)), to sequences that are
756 taxonomically annotated such as NCBI's GenBank nucleotide database (Benson *et al.* 2013),
757 BOLD (Ratnasingham & Hebert 2007), or to self-generated reference databases from Sanger-
758 sequenced DNA (Olds *et al.* 2016; Sønstebø *et al.* 2010; Willerslev *et al.* 2014). Then, to at least
759 partially corroborate the resulting list of taxa, comparisons are made with conventional physical,
760 acoustic, or visual-based survey methods conducted at the same time or compared with historical
761 records from surveys for a location (see Table 1).
762
763 The difference in source material between community DNA and eDNA, therefore, has
764 distinct ramifications for interpreting the scale of inference for time and space about the
765 biodiversity detected. From community DNA it is clear that the individual species were found in
766 that time and place, but for eDNA, the organism which produced the DNA may be upstream
767 from the sampled location (Deiner & Altermatt 2014), or the DNA may have been transported in
768 the feces of a more mobile predatory species (e.g., birds depositing fish eDNA, Merkes *et al.*
769 2014) or was previously present, but no longer active in the community and detection is from
770 DNA that was shed years to decades before (Yoccoz *et al.* 2012). The latter means that the scale
771 of inference both in space and time must be considered carefully when inferring the presence for
772 the species in the community based on eDNA.

Box 2. Basic bioinformatic pipeline for eDNA metabarcoding for plants and animals

Bioinformatic processing of sequence data is one of the most critical aspects of eDNA metabarcoding studies, helping to substantiate research findings, following field and lab work components. Standardization of bioinformatics in a 'pipeline' can ensure quality and reproducibility of findings; however, some level of customization is required across studies. Customization is needed to compensate for advances in sequencing technology, software workflows, and the question being addressed. Therefore, taking raw read data and turning it into a list of taxa, requires multiple quality assurance steps – some necessary, others optional. Reaching an absolute consensus for the approaches and software used is not necessary as these will always be in flux, but here we advise careful consideration of the following pre-processing steps *at a minimum* for HTS data before embarking on further analyses (e.g., for biodiversity estimates and statistical significance). We focus primarily on processing Illumina generated data sets and therefore if the technology is different, many of the bioinformatic tools highlighted and advice is transferable to pre-processing of data produced on other platforms, but may be different.

Terms:

Chimeras: PCR artefacts made of two or more combined sequences during the extension step of PCR amplification.

Phred quality score: Quality scoring per nucleotide for Illumina sequencing providing the probability that a base call is incorrect.

Sequence merging: Combining forward (R1) and reverse (R2) reads from paired – end (PE) sequencing, using criteria such as minimum overlap or quality score.

Sequence trimming: The process of cutting / removing the beginning or end of sequencing reads. Can be performed either by searching for a specific sequence (removal of adaptors, indexes and primers) or based on quality score.

Singletons: MOTUs that appear only once in the data are likely to be rare taxa, false positives, low level contamination, or unremovedchimeras, and should be treated with appropriate consideration.

Primer – adaptor trimming. Preliminary steps of bioinformatics processing include de-multiplexing of the samples based on the indices used (unique nucleotide tags incorporated into raw sequence data) and trimming (i.e., removal) of the adaptor sequences. The adaptors are specific DNA fragments which are added during library preparation for ligation of the DNA strands to the flow cell during Illumina sequencing. Additionally, the index sequences themselves and the primer sequences should be trimmed (e.g. using software such as Cutadapt, Trimmomatic, QIIME), allowing either zero or a low level of mismatch between the exact sequence of the primer or index and the observed reads.

Merging or end trimming. Sequences from Illumina runs tend to drop in quality towards the 3' end of the reads, as phasing leads to increased noise (and lower signal) in later chemistry cycles. Thus, the quality score of reads should be reviewed to allow informed decisions on the appropriate length of end trimming (single – end runs), merging (paired end runs) and subsequent sequence quality filters. Visualizing the quality scores from raw reads or de-multiplexed sequences (using software like FastQC) will help with the selection of downstream quality cut-off levels.

When paired end (PE) sequencing is used for an amplicon of suitable size, the forward (R1) and reverse (R2) reads should be combined (merged) to form the complete amplicon. Using merged sequences improves accuracy since the lower quality bases at the tail ends of individual reads can be corrected based on the combined reads. Here, the minimum overlap for R1 and R2 reads should be specified and 'orphan' reads with little or no overlap between forward and reverse pairs can be discarded. Inspection of the quality scores, as mentioned above, can provide an estimate of optimal parameters for merging of R1 and R2 reads. Even though a specific consensus does not exist yet, in many cases an overlap of at least > 20bp is selected (Deiner *et al.* 2015; Gibson *et al.* 2015).

Quality filtering. For most HTS platforms, a Phred score is calculated and subsequently used to determine the maximum error probabilities (Bokulich *et al.* 2013). Selected strategies include filtering based on a lower Phred score cut-off, usually set at least above 20 or 30 (Bista *et al.* 2017; Elbrecht & Leese 2015; Hänfling *et al.* 2016). Quality filtering can also be performed based on maximum error (maxee) probability, which is also derived from Phred scores. The lower the maximum error, the stricter the cut-off. Selection of a maximum error filtering level of 1 or 0.5 is common in macro-organism studies (Bista *et al.* 2017; Pawlowski *et al.* 2014; Port *et al.* 2016). Additionally, in the case of single-end sequencing, or when long amplicons without sufficient overlap of the forward and reverse reads are used, it is advised that trimming should be performed from the appropriate end. It is often the case that reads are trimmed to a common length, which facilitates alignment downstream and minimizes miscalled bases since a merging step cannot be used.

Removing short reads. Many studies also select to remove short reads from the dataset before clustering since the presence of high length variation could influence the clustering process (see USEARCH manual, Edgar 2010). These sequences could result from sequencing of primer dimers which have not been removed (Pawlowski *et al.* 2014). Different studies select a variety of minimum length reads, from very short 20bp (Valentini *et al.* 2016), to medium 60 – 80 bp (Pawlowski *et al.* 2014; Shaw *et al.* 2016) and up to 100 bp (Bista *et al.* 2017; Gibson *et al.* 2015; Hänfling *et al.* 2016; Pawlowski *et al.* 2014). Note that some de-multiplexing or quality filtering workflows may automatically set a minimum sequence length when processing input data and it is advisable to check whether such a parameter is included by default.

Removing singletons and chimeras. Important steps after MOTU clustering involve removal of singletons and chimeras. Chimeras are by-products of the PCR amplification process from two or more parental sequences (chimeric), most commonly produced through an incomplete extension step (Edgar *et al.* 2011). It has been shown that when unique reads, such as chimeras and singletons, are withheld in analysis, the estimation of diversity can be severely inflated (Kunin *et al.* 2010). The nature of the chimeric sequences, which can be present as high quality reads, does not enable their removal directly through quality based end-trimming (Coissac *et al.* 2012). Removal of chimeras can be performed either *de novo* or based on a reference database. Most common practice to date is the *de novo* method since a sufficient reference database may not be available. Despite the variation in software used such as UCHIME (Edgar *et al.* 2011), obitools (Boyer *et al.* 2016), or ChimeraSlayer (Haas *et al.* 2011), there is a consensus regarding the importance of removing chimeras and singletons as a minimum quality control for bioinformatics pipeline.

Abundance filtering. In addition to quality filtering based on quality scores and removal of chimeras and singletons, many studies also employ further filtering for removal of low abundance sequences (Murray *et al.* 2015). This step arises from the need to control for laboratory contamination or because of cluster contamination on the flow cell (unique to Illumina platforms) (Olds *et al.* 2016).

The process of applying abundance filtering requires setting an MOTU abundance threshold by which MOTUs are only retained in analysis if their relative abundance is higher than the selected threshold (Bokulich *et al.* 2013). Selection of a threshold varies between studies and there is no generally accepted definition of what constitutes an insufficiently abundant read (Murray *et al.* 2015), perhaps with the exception of singletons. Abundance filtering may be applied minimally or avoided entirely, especially if stringent quality trimming parameters are applied to raw reads and detection of "rare" MOTUs is an important aspect of a study (Bokulich *et al.* 2013). Another option that could be used involves selection of a threshold based on availability of empirical data as was done in Valentini *et al.* (2016). An increasing number of studies have employed the sequencing of positive controls to establish a threshold level (Hänfling *et al.* 2016; Port *et al.* 2016; Stoeckle *et al.* 2017). Technical replicates can also be used to assess consistency as was shown to be effective with assessing omnivore diets (De Barba *et al.* 2014).

Using a positive control defined error level works by identifying the abundance of sequences in the control sample that belong to non-target taxa and can be the result of errors such as contamination. Furthermore, the distribution of *phiX* reads assigned to target samples has been used to investigate the presence of "tag-jumps" (Schnell *et al.* 2015a) and mis-assigned reads during de-multiplexing (Hänfling *et al.* 2016; Olds *et al.* 2016). The exact mechanisms for mis-assignment of reads remain unknown, but increasingly many studies are reporting this error to be between 0.01 and 0.03 % of reads (Hänfling *et al.* 2016; Olds *et al.* 2016; Stoeckle *et al.* 2017). Adjustments for this include use of a threshold approach based negative and/or positive controls and removes a low number of reads from any given sample. The issue of abundance filtering most significantly causes uncertainty in low abundance MOTUs and will continue to be a problem for detection of rare species. Therefore, to avoid negative impacts to scientific insights or management decisions, careful consideration and transparency regarding how technical artifacts are dealt with during bioinformatic data analysis is needed until these artifacts are well understood.

Recording removed data. For all quality control steps the data removal should be transparent. Often studies report the total number of sequences obtained, but then rarely show how each quality filtering step affects the number of sequences used in testing ecological hypothesis nor do researchers provide the subset of sequences that were retained or omitted. Deleting data without a clear justification does not allow transparency. Therefore, including a supplemental table in eDNA metabarcoding studies showing the number of sequences remaining after each filtration step is advised and archiving the subset of reads retained after each filtering step on a platform such as Dryad (http://datadryad.org/) or archiving the exact pipeline with version control information on a platform such as GitHub (https://github.com/) will allow for greater transparency and reproducibility of quality filtering.

Box 3: How to transform reads from HTS platforms into measures of richness

MOTU clustering. While this step is not always necessary and depends on the target set of taxa (Lacoursière-Roussel *et al.* 2016), the amplicon length sequenced (Deiner *et al.* 2016), and completeness of the reference database (Chain *et al.* 2016), clustering of sequencing reads into MOTUs is often performed prior to taxonomic assignment. MOTU clustering is the process whereby multiple reads are grouped according to set criteria of similarity based on an initial seed (Creer *et al.* 2016; Egan *et al.* 2013). Here, a centroid sequence is selected and depending on the set radius or similarity cut-off, closely related sequences are grouped under each centroid sequence (USEARCH, Edgar 2010). The level of similarity selected depends on the study and taxon used, based on the knowledge of intraspecific diversity of the studied taxon. Commonly used cut-offs range from 97% to 99% (Bista *et al.* 2017; Fahner *et al.* 2016; Olds *et al.* 2016). For example, the cut-off selected could depend on known levels of intraspecific diversity of the studied taxon, which could be estimated from an existing reference database. Some commonly used clustering algorithms include USEARCH (Edgar 2010), VSEARCH (Rognes *et al.* 2016), CROP (Bayesian clustering algorithm) (Hao *et al.* 2011), swarm (Mahé *et al.* 2014), and mothur (an alignment-based clustering method, Schloss *et al.* 2009).

Taxonomic assignment. Identification of HTS reads is achieved through a comparison of anonymous MOTU clusters/centroid sequences or direct comparisons of reads remaining after quality filtering against a reference database. Depending on the taxon of study and the marker used, the reference database may consist of publicly available sequences or study-generated reference sequences.

The challenges of taxonomic assignment have been the subject of a considerable literature so we only briefly discuss this important aspect of the bioinformatics pipeline (e.g., Bazinet & Cummings 2012). A number of different approaches have been suggested including assignment based on sequence similarity via alignment programs like BLAST or similarity searches using Hidden Markov Models such as jMMOTU (Jones *et al.* 2011), MG-RAST (Glass *et al.* 2010), sequence composition and machine learning approaches (e.g., RDP (Wang *et al.* 2007), TACOA (Diaz *et al.* 2009)), phylogenetic placement (e.g., pplacer Matsen *et al.* 2010), probabilistic taxonomic placement (e.g., PROTAX (Somervuo *et al.* 2016; Somervuo *et al.* 2017), minimum entropy decomposition (e.g., oligotyping Eren *et al.* 2015), MEGAN (Huson *et al.* 2007), and ecotag (Boyer *et al.* 2016). A number of widely used programs use combinations of these methods, for example, the program SAP (Munch *et al.* 2008) uses BLAST searches of the NCBI database and phylogenetic reconstruction to establish taxonomic identity of query sequences. Most of these methods and various derivatives are nicely discussed and compared by Bazinet and Cummings (2012). Two major determinants of the utility of these different approaches are the specific eDNA markers and the breadth and resolution of reference databases. Some markers have better representation in available databases and greater coverage of relevant species diversity. Taxonomic assignment using the BLAST algorithm (Camacho *et al.* 2009) is commonly used and depending on the study, different selection criteria are specified, such as e-value, maximum ID or length of matching sequence, number of top hits selected, etc. Caution is warranted in strictly relying on this approach, since errors in the curation of sequences in publicly available databases can propagate through the analysis and lead to misidentification of sequences. Ideally, a combination of approaches is used and when feasible the resultant species

assignments should be vetted with independent data based on the known distribution and ecology of the species.

Diversity analysis. The goal of most eDNA metabarcoding studies is to accurately characterize the species richness of the community under study. Calculation of diversity indices using appropriate software allows modeling and ecological association of sequencing results. Important considerations when attempting ecological associations include appropriate data standardization to account for variations in sequencing depth and the careful selection of diversity indexes. The most common assessments include alpha-diversity (rarefaction, visualization of taxonomic profiles), and beta-diversity (Principal Components/Coordinates Analysis, NDMS ordination, etc.), prior to hypothesis testing via downstream statistical analysis.

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1478 Table 1: Representative studies comparing richness estimates with traditional sampling or historical data for a geographic location to

1479 that of eDNA metabarcoding.

Habitat	Macro-organism taxonomic focus	eDNA sample type	Traditional sampling method	eDNA efficacy finding*	Authors	Year
Air	Plants	air pollen trap	morphological identification	Better taxonomic resolution	Kraaijeveld *et al.*	2015
Freshwater	Fish	flowing water	depletion-based electro fishing	Higher diversity	Olds *et al.*	2016
Freshwater	Invertebrates	flowing water	kicknet in stream and historical data	Higher diversity	Deiner *et al.*	2016
Freshwater	Fish	stagnant water	gill-net, trapping, hydroacoustics, analysis of recreational anglers' catches	Complementary	Hänfling *et al.*	2016
Freshwater	Reptiles, amphibians	stagnant water	species distribution model based on historical data (i.e. distribution range and habitat type)	Increase species distribution knowledge	Lacoursière-Roussel *et al.*	2016
Freshwater	Amphibians, fish	stagnant water; flowing water	amphibians: visual encounter survey, mesh hand-net; Fish: electrofishing, and/or netting protocols (fyke, seine, gill)	Greater detection probability	Valentini *et al.*	2016
Freshwater	Amphibians, fish, mammals, invertebrates	stagnant water; flowing water	active dip-netting, fresh tracks or scat, electrofishing with active dip-netting	Complementary	Thomsen *et al.*	2012
Freshwater	Fish	stagnant water; flowing water; surface sediment	fyke net	Higher diversity	Shaw *et al.*	2016
Freshwater	Invertebrates	water column; surface sediment	sediment collected using a Van Veen grab	Higher diversity	Gardham *et al.*	2014

Freshwater	Fish / Diptera	Surface and bottom water column	Long-term data, electro fishing (fish) and emerging traps (Diptera) at time of eDNA sampling	Higher diversity compared to sampling but lower diversity compared to long-term data	Lim *et al.*	2017
Marine	Fish	Surface and bottom water column	Long term observation	Complementary	Yamamoto *et al.*	2017
Marine	Fish	Bottom water column	Trawl catch data	Similar Family richness	Thomsen *et al.*	2016
Marine	Fish	water column	scuba diving	Higher diversity	Port *et al.*	2015
Terrestrial	Plants	honey	melissopalynology (i.e. pollen grains retrieved from honey are identified morphologically)	Complementary	Hawkins *et al.*	2015
Terrestrial	Mammals, plants	midden pellets	historical surveys	Higher diversity	Murray *et al.*	2012
Terrestrial	Mammals	saliva	local knowledge (i.e. physical evidence) and camera data	Complementary	Hopken *et al.*	2016
Terrestrial	Birds, invertebrates, plants	top soil	invertebrates: leaf litter samples & pitfall traps; reptiles: pitfall traps and under artificial ground covers; birds: distance sampling method; plants: above-ground surveys	Complementary for plants & invertebrates	Drummond *et al.*	2015
Terrestrial	Earthworms	top soil	irrigated quadrats with 10 L of allyl isothiocyanate solution and hand collected emerging worms	Complementary	Pansu *et al.*	2015
Terrestrial	Plants	top soil	historical surveys	Complementary	Jørgensen *et al.*	2012
Terrestrial	Plants	top soil	above-ground surveys	Complementary and better taxonomic resolution	Yoccoz *et al.*	2012

Terrestrial	Vertebrates	top soil	local knowledge from safari parks, zoological gardens and farms; visual observations; historical surveys	Complementary	Andersen *et al.*	2012

* Complementary means the two survey methods detected different diversity, but does not exclude that some of the diversity was detected by both methods. Higher diversity means the study found more diversity was detected compared to conventional, but does not exclude that some of the diversity was *not* detected by both methods. Better taxonomic resolution means that sequence based identifications could be resolved to a lower taxonomic rank compared with the conventional method.

Figure legends

Figure 1: Environmental DNA sample types have different spatial and temporal scopes of inference from different habitats. Consider each sample type as a single sample from that environment. Placement of a sample type in a quadrant is not quantitive, but represents a common scale at which it has been used. Dashed arrows indicate the potential for a sample type to confer information at multiple scales of inference, but additional research to quantify these possibilities in needed.

Figure 2: Challenges for estimating abundance from environmental DNA metabarcoding. For simplicity, assume one DNA molecule depicted in the pond is equal to one organism and colors represent different species. Additionally for this example, assume that sampling is no biased (i.e., DNA copies are sampled in their true abundance), that boxes surrounding DNA molecules represent 1 uL and one DNA molecule represents 1 ng of DNA. Thus, values illustrated show the effect of primer bias, sub-sampling and their combination on the ability to estimate abundance.

Figure 3: Important guiding questions for consideration in the design and implementation phases of an environmental DNA metabarcoding study.

Figure 4: Opportunities and challenges of using environmental DNA as a tool for assessing community structure in different fields of study. The tool is reliant on a foundation (blue half circle) of continued research to improve technological aspects and continued development of DNA-based reference libraries for the identification of sequences found in the environment.

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Author contributions

K.D. outlined and edited the review. All authors contributed at least one section of primary writing and contributed to editing of the manuscript. K.D., H.M.B, and E.M. synthesized sections and drafted figures.

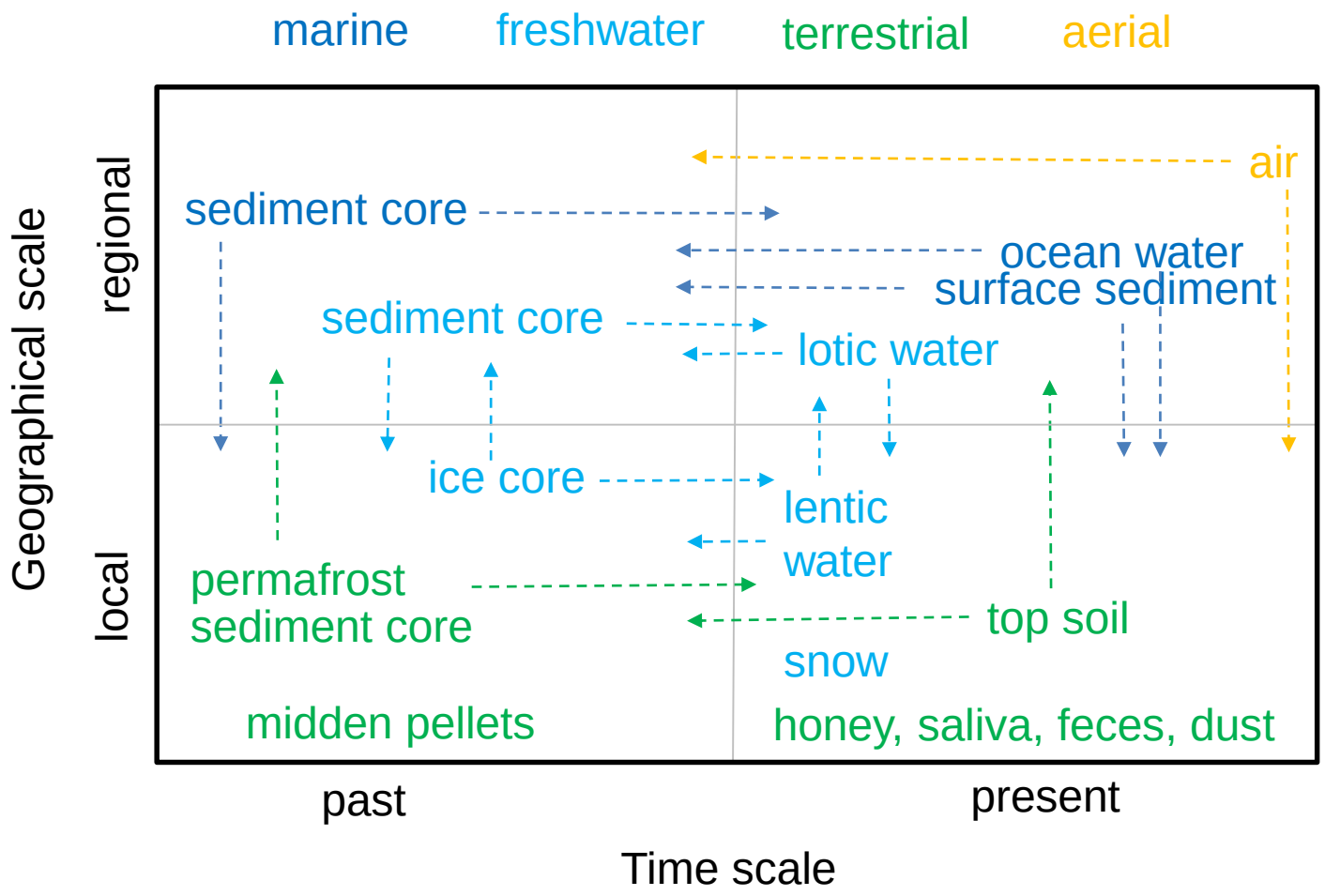
Data Accessibility

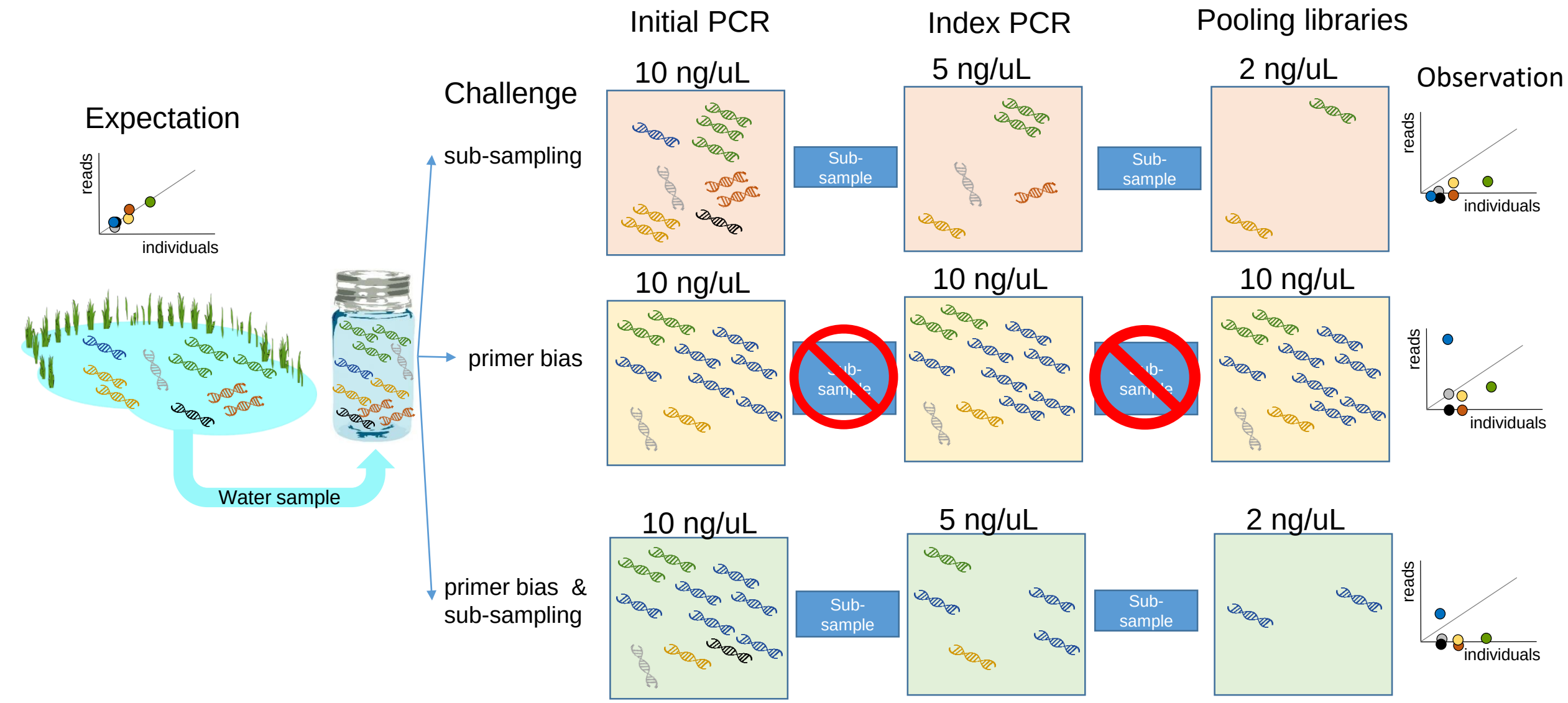
No data are associated with the manuscript

Supplemental Material

Table S1: Reviews about use of environmental DNA for species detection

Table S2: Review of primers used in eDNA metabarcoding


marine
freshwater
terrestrial
aerial
Geographical scale
regional
local
air
sediment core
ocean water
surface sediment
sediment core
lotic water
ice core
lentic
water
permafrost
sediment core
top soil
snow
midden pellets
honey, saliva, feces, dust
past
present
Time scale


Expectation
reads
individuals
Water sample
Challenge
sub-sampling
primer bias
primer bias &
sub-sampling
Initial PCR
Index PCR
Pooling libraries
Observation
10 ng/uL
5 ng/uL
2 ng/uL
Sub-
sample
10 ng/uL
10 ng/uL
10 ng/uL
10 ng/uL
5 ng/uL
2 ng/uL
reads
individuals

WORKFLOW

Study design

Basic science or applied? (e.g., environmental biomonitoring)

What is your study goal?
- **presence/absence**
- **diversity assessment**
- **absolute quantification**

What taxa will you target?

Is the scale of inference for your sample type appropriate to your question?

Can you compare complementary data types? (e.g. traditional vs. eDNA)

Does your sampling/ replication scheme provide good statistical power?

In the field

What type of sample is needed? (water, soil, air)

What metadata should you collect?

How many replicates will you collect?

Does your sampling protocol minimize/ control for :
- **contamination** (e.g., positive and negative controls)
- **any known biases** (e.g., inhibitors, sample volume)

In the laboratory

Sample Handling Phase

What extraction method? (physical vs. chemical)

How much sample?

What locus and primers?

Do you need to generate reference sequence data?

Are technical replicates needed?

What library preparation method will you use?

How many samples will you index and pool?

What sequence depth is needed per sample ?

What read length will you use?

DNA Processing Phase

What sequencing platform will you use?

Do you need paired end sequencing?

Have you included appropriate quality assurances? (e.g., mock community, qPCR, bioanalyzer traces)

Does your laboratory protocol minimize/ control for:
- **contamination** (e.g., positive and negative controls)
- **any known biases** (e.g., primer bias, coverage, taxonomic resolution)

At the keyboard

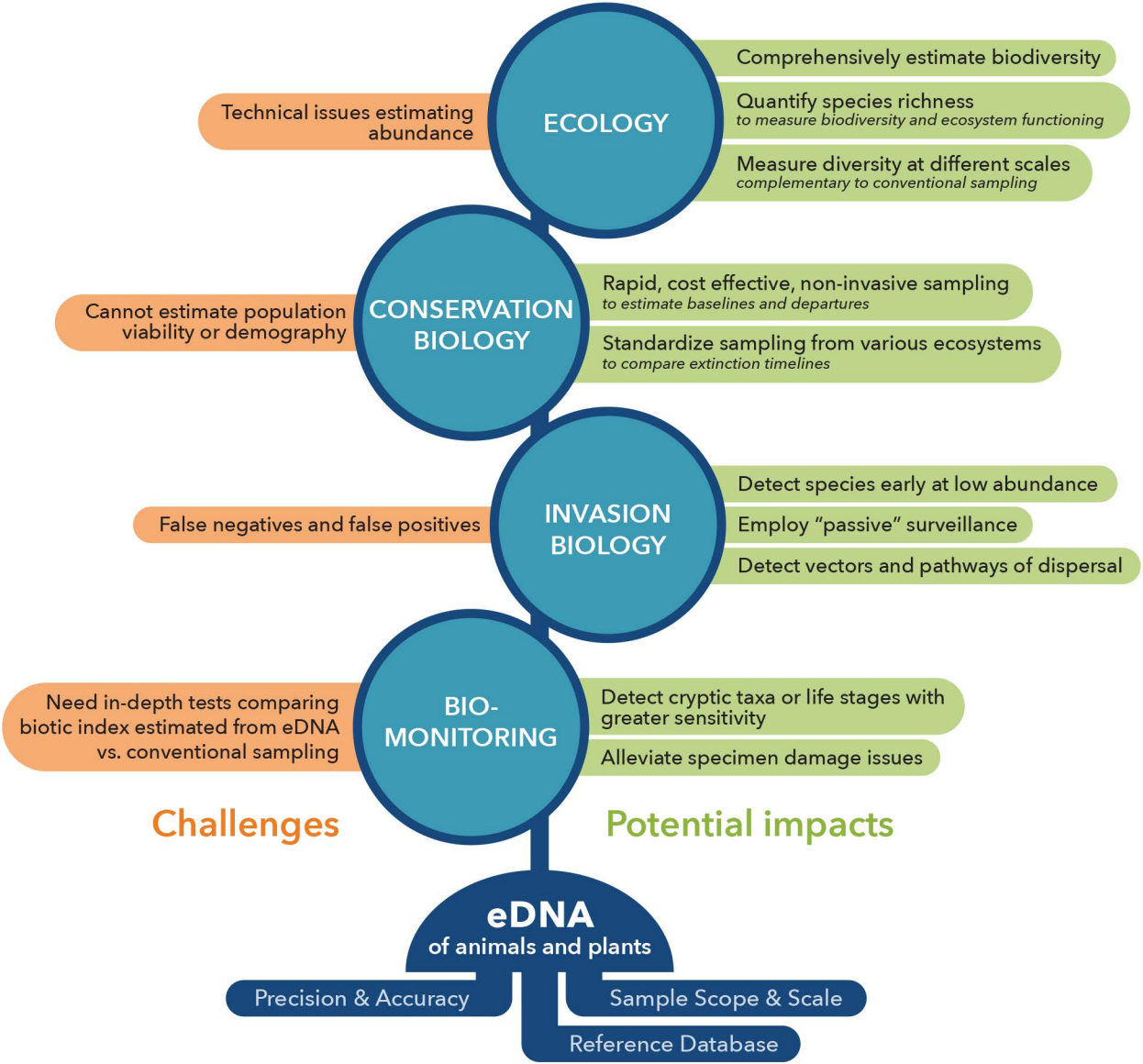
How complete is the reference database?

Do you have adequate sequencing coverage across samples?

Are you using appropriate choices for software tools, parameters?

Are your biological conclusions upheld using alternative parameters and workflows?

Are you including appropriate quality filtering of your data? (see Box 2)


ECOLOGY
Technical issues estimating abundance
Comprehensively estimate biodiversity
Quantify species richness
to measure biodiversity and ecosystem functioning
Measure diversity at different scales
complementary to conventional sampling
CONSERVATION BIOLOGY
Cannot estimate population viability or demography
Rapid, cost effective, non-invasive sampling
to estimate baselines and departures
Standardize sampling from various ecosystems
to compare extinction timelines
INVASION BIOLOGY
False negatives and false positives
Detect species early at low abundance
Employ "passive" surveillance
Detect vectors and pathways of dispersal
BIO-MONITORING
Need in-depth tests comparing biotic index estimated from eDNA vs. conventional sampling
Detect cryptic taxa or life stages with greater sensitivity
Alleviate specimen damage issues
Challenges
Potential impacts
eDNA
of animals and plants
Precision & Accuracy
Sample Scope & Scale
Reference Database